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Interactive report

Ibogaine acts at the nicotinic acetylcholine receptor to inhibit catecholamine release¹

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Abstract

In an effort to determine mechanisms of action of the putative anti-addictive agent ibogaine, we have measured its effects on catecholamine release in a model neuronal system, cultured bovine chromaffin cells. Various modes of stimulating catecholamine release were used including nicotinic ACh receptor activation, membrane depolarization with elevated K⁺ and Na⁺ channel activation with veratridine. In addition, because ibogaine has been reported to interact with kappa opioid receptors, we tested whether kappa receptor antagonists could reverse ibogaine's effects on catecholamine release. Ibogaine, at low concentration (< 10 μ M) was found to selectively inhibit nicotinic receptor-mediated catecholamine release, while having no significant effect on release evoked by either veratridine or membrane depolarization with elevated K⁺. The inhibitory actions of ibogaine and the kappa agonists were not reversed by preincubation with the opioid antagonists nor-binaltorphimine or naltrexone, suggesting that these inhibitory effects are not mediated by the kappa opioid receptor. The effects of low dose (10 μ M) ibogaine were rapidly reversible, while the inhibitory effects of higher ibogaine doses persisted for at least 19 h following ibogaine washout. The results provide evidence for a mechanism of action ibogaine at the nicotinic ACh receptor. The results are consistent with a model in which the initial high transient brain concentrations (100 μ M) of ibogaine act at multiple cellular sites and then have a selective action at the nicotinic ACh receptor cation channel following its metabolism to lower brain concentrations. The present findings are relevant to potential anti-addictive actions of ibogaine and to the development of drugs to combat nicotine addiction. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Ibogaine mechanism; Nicotinic acetylcholine receptor; Anti-addictive agent; Chromaffin cell; Catecholamine release

1. Introduction

Ibogaine, an indole alkaloid derived from the West African shrub, *Tabernanthe iboga*, has generated much interest in recent years as a putative anti-addictive substance with long term action [40]. Remarkable claims have been made, based on anecdotal evidence, that ibogaine interrupts addiction in humans to opiates, cocaine, amphetamine, alcohol and nicotine [25–28]. Ibogaine purportedly exerts a long-term inhibitory effect on drug-dependent behavior following a short course of exposure to the drug — a single dose having been claimed to suspend addictive behavior for up to six months [25,26]. Animal studies indicate the ability of ibogaine to attenuate self-administration of opiates and cocaine by rats for periods of days to weeks [10,17–19]. Ibogaine has also been reported to

inhibit catecholamine release stimulated by a variety of addictive substances including opiates, cocaine and nicotine, both in the brain's mesolimbic pathway [2,30,32] and in neuronal cell cultures [42,43]. Ibogaine's effects on catecholamine release may be relevant to its cellular mechanism of action since it has long been held that the action of many addictive drugs is mediated by an enhancement of mesolimbic dopamine release [16,23].

Recent studies have sought to elucidate the cellular sites and mechanism by which ibogaine interferes with the catecholamine release response. Radioligand binding studies indicate that ibogaine interacts with a number of receptor and ion channel sites including kappa and mu opioid receptors, sigma receptors, NMDA receptor channels and voltage-gated sodium channels [3,12,15,29,39,40,46]. Several papers have appeared during the last two years suggesting the nicotinic acetylcholine receptor as a potentially important site at which ibogaine may alter catecholamine release and exert an anti-addictive action. We have reported that ibogaine selectively inhibits nicotinic receptor-

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mediated catecholamine release in chromaffin cell cultures and have suggested a direct interaction of ibogaine with the nicotinic receptor channel [42]. Consistent with this finding are recent reports from other laboratories showing that ibogaine inhibits several nicotinic agonist-mediated responses including: (1) nicotine-evoked mesolimbic dopamine release in rat brain [2,32]; (2) carbamylcholine-induced ^{22}Na influx through nicotinic receptor channels in rat pheochromocytoma PC12 cells and human medulloblastoma TE671 cells [1]; and (3) epibatidine-induced antinociception in mice [1].

The objective of the present study is to further clarify the mechanisms of action of ibogaine, focusing on nicotinic acetylcholine receptors, kappa-opioid receptors and Na channels as possible sites of action in cultured chromaffin cells. We show that ibogaine's inhibitory action on catecholamine release at low ibogaine doses (1–10 μM) is mediated by the nicotinic receptor, and not by an action at Na channels or kappa opioid receptors. Reversibility and persistence of ibogaine's inhibitory actions, following ibogaine washout, is shown to be dependent on the concentration of ibogaine to which the cells are exposed. Because ibogaine has been reported to interact with kappa opioid receptors, the ability of the opioid antagonists norbinaltorphimine (norBNI) and naltrexone to reverse ibogaine's inhibitory effects on catecholamine release were tested. A preliminary report on this work has recently been published [43].

2. Materials and methods

2.1. Materials

Collagenase type I was obtained from Worthington Biochemical (Freehold, NJ); [^3H]Norepinephrine from DuPont-New England Nuclear; deoxyribonuclease I, norepinephrine hydrochloride, bovine serum albumin, acetylcholine chloride, veratridine, HEPES and tetrodotoxin from Sigma Chemical, St. Louis, MO; ibogaine and naltrexone from RBI, Natick, MA; Penicillin, streptomycin, gentamycin, fungizone and fetal bovine serum from GIBCO, Grand Island, NY. norBNI was provided by S. Archer, Rensselaer Polytechnic Institute, Troy, NY.

2.2. Isolation and primary culture of bovine adrenal chromaffin cells

Adrenal chromaffin cells were isolated with minor modifications of a previously described collagenase disaggregation procedure [6,21]. Four bovine adrenal glands were perfused with 500 ml perfusion buffer (145 mM NaCl, 5.4 mM KCl, 1.0 mM NaH_2PO_4 , 11.2 mM glucose, 15 mM HEPES, pH 7.4) and subsequently injected with 5 ml/gland of concentrated collagenase solution (0.25% collagenase type I and 0.007% DNase I in perfusion buffer).

The glands were incubated for 15 min at 37°C, and the injection was repeated twice. The digested medulla was removed and minced, and incubated with collagenase solution (0.05% collagenase type I and 0.001% DNase I in perfusion buffer) for 1 h at 37°C in a shaking water bath. Isolated chromaffin cells were obtained by filtration through a 250 μm mesh, and harvested by centrifugation at $478 \times g$. Cells were washed several times with perfusion buffer, resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum, and filtered through a 100 μm mesh. Cell viability, determined by erythrosin B vital dye exclusion, was > 90%.

Freshly isolated chromaffin cells were maintained in primary culture as we have previously described [6]. Cells were suspended in DMEM supplemented with 10% heat inactivated fetal bovine serum, Cytosar U (10 μM), 5'-fluorodeoxyuridine (100 μM), fungizone (2.5 $\mu\text{g}/\text{ml}$), penicillin/ (200 U/ml), streptomycin (200 $\mu\text{g}/\text{ml}$) and gentamicin (40 $\mu\text{g}/\text{ml}$), and plated on 24-well plates at a density of 3×10^5 cells per well. Cells were incubated at 37°C in a water jacketed CO_2 incubator with 5% CO_2 /95% air. Cells were generally used within 10 days of plating.

2.3. Catecholamine secretion

Catecholamine secretion from chromaffin cells was measured as release of preloaded [^3H]norepinephrine ([^3H]NE) as previously described [6,34]. Cells were loaded with [^3H]NE (2 $\mu\text{Ci}/\text{ml}$)/NE (total concentration = 0.5

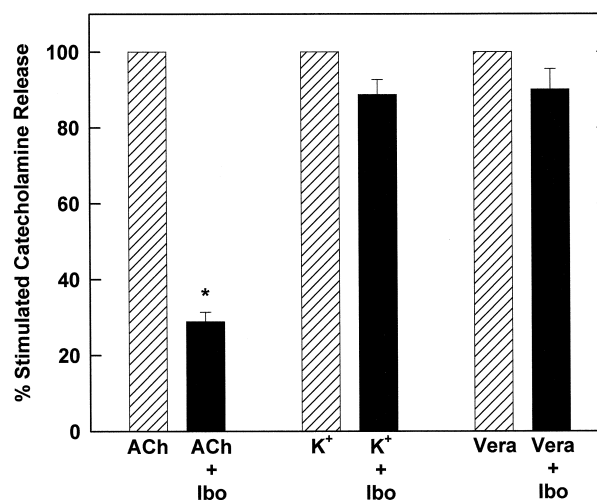


Fig. 1. Selective effects of ibogaine on nicotinic receptor-mediated catecholamine release. Chromaffin cells were preincubated with ibogaine (10 μM) for 10 min and subsequently challenged with either acetylcholine (ACh, 30 μM), elevated K^+ (70 mM), or veratridine (100 μM) in the presence of ibogaine for 10 min at 37°C. Results are expressed as % of stimulated control catecholamine release in the absence of ibogaine. The data represent the mean $\pm$ SEM of 4–8 independent experiments each performed in triplicate. * indicates that the difference between ACh and (ACh + Ibogaine) was significant with $p < 0.01$ by Paired Student's t -test.

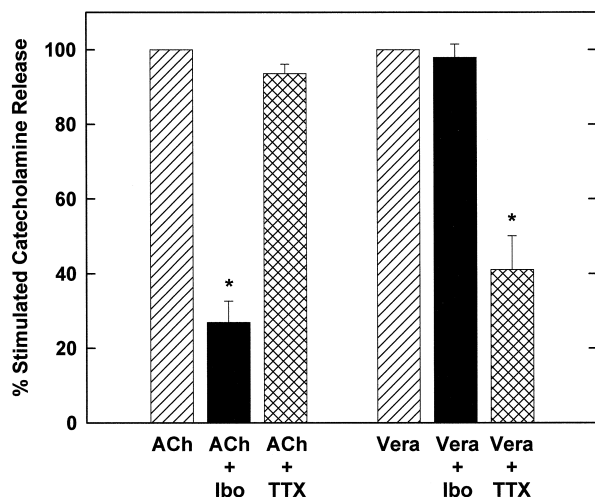


Fig. 2. Comparison of the effects of ibogaine and tetrodotoxin on catecholamine release evoked by acetylcholine and veratridine. Chromaffin cells were preincubated with either ibogaine (10 μ M) or tetrodotoxin (1 μ M) (TTX) for 10 min prior to stimulation of the cells with acetylcholine (30 μ M) or veratridine (100 μ M) for 10 min at 37°C. Results are expressed as % of stimulated control catecholamine release in the absence of ibogaine or TTX. The data represent the mean $\pm$ SEM of 4 independent experiments each performed in triplicate. * indicates that difference between ACh and (ACh + Ibogaine), and between veratridine and (veratridine + TTX) were significant with $p < 0.01$ by Paired Student's t -test.

μ M) in HEPES buffer solution (137 mM NaCl, 4.4 mM KCl, 3.6 mM NaHCO_3 , 1.2 mM KH_2PO_4 , 10 mM glucose, 1.2 mM MgSO_4 , 2.2 mM CaCl_2 , 0.5% bovine serum albumin (BSA) and 5 mM HEPES, pH 7.4) plus 0.1% ascorbic acid for 1 h at 37°C. Extracellular radioactivity

was removed by washing at least three times at 15 min intervals with HEPES buffer. Where specified, cells were preincubated in HEPES buffer containing either ibogaine, tetrodotoxin, naltrexone or norBNI for 10 min at 37°C. The resulting supernatant was collected to determine the effect of these agents on basal catecholamine release. Cells were subsequently stimulated for 10 min with 30–100 μ M acetylcholine (ACh), 100 μ M veratridine or 70 mM KCl in the presence of the appropriate agent in HEPES buffer. For experiments aimed at determining the reversibility and persistence of ibogaine effects, cells were preincubated with the indicated concentration of ibogaine for 120 min. The ibogaine was then either maintained or washed out of the medium for the indicated period of time prior to challenge with ACh. Stimulated catecholamine release was quantitated by liquid scintillation counting of the secreted [^3H]NE in the extracellular medium. Catecholamine release was normalized in terms of percentage of total starting intracellular [^3H]NE, determined by lysis of the cells with 8% trichloroacetic acid (TCA). 30–100 μ M ACh and 100 μ M veratridine typically stimulated the release of 20–30% of total cell catecholamines. 70 mM KCl evoked the release of 15–20% of total cell catecholamines.

3. Results

The effect of ibogaine on various modes of stimulated catecholamine release was examined in cultured chromaffin cells. Preexposure of cells for 10 min to 10 μ M

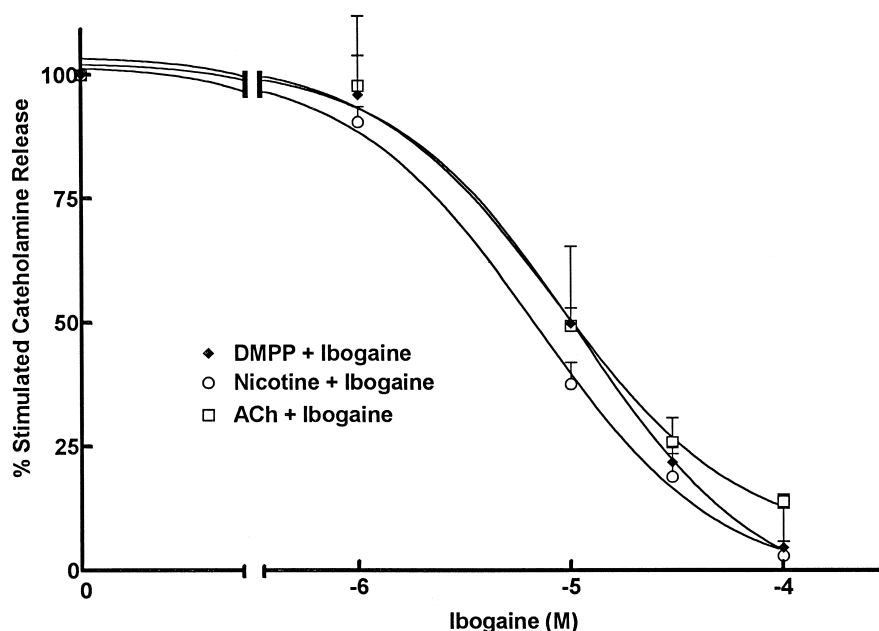


Fig. 3. Dose dependence and reversibility of Ibogaine effects on catecholamine release evoked by different nicotinic receptor agonists. Chromaffin cells were preincubated with varying concentrations of ibogaine for 10 min. Ibogaine was removed out during the subsequent 10 min stimulation with each of the following nicotinic receptor agonists: ACh (100 μ M), nicotine (10 μ M), DMPP (10 μ M). Results are expressed as % of maximum stimulated catecholamine release. Results were normalized by subtracting basal release, which was typically $< 2\%$ of total cellular catecholamine content.

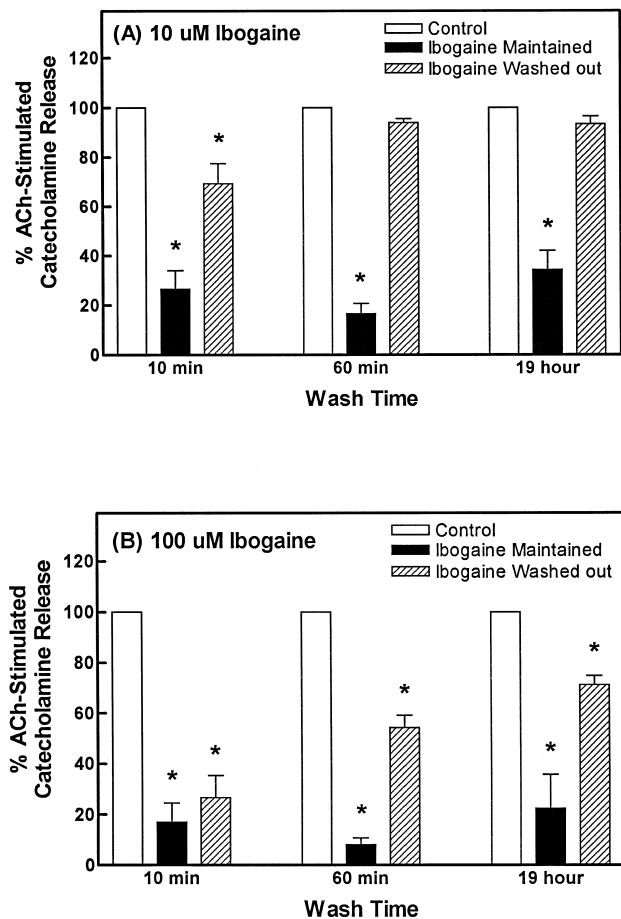


Fig. 4. Time course of reversal of ibogaine inhibition of catecholamine release following ibogaine washout. Chromaffin cells were preincubated with either 10 μ M (Panel A) or 100 μ M (Panel B) ibogaine for 120 min, and subsequently subjected to a wash period of varying duration (10 min to 19 h) in which ibogaine was either present (■) or washed out (hatched). Finally, cells were stimulated with ACh for 10 min in the presence (■) or absence (hatched) of ibogaine. Results are expressed as % of control ACh stimulated catecholamine release in the absence of ibogaine. Data represent the mean $\pm$ SEM of 4–5 independent experiments each performed in triplicate. * represents significant difference ($p < 0.05$) between control ACh stimulated release (open bar) and indicated experimental condition.

ibogaine resulted in a 70% inhibition of ACh-stimulated catecholamine release (Fig. 1). In contrast, release evoked by either depolarization with high KCl (70 mM) or the Na channel agonist, veratridine (100 μ M) was not significantly affected by 10 μ M ibogaine. Since the primary pathway of ACh stimulation in bovine chromaffin cells is via nicotinic ACh receptors, these results suggest the nicotinic receptor as a site of action of ibogaine.

Ibogaine has been reported to compete with batrachotoxin-B for binding to voltage-gated sodium channels [15,46]. Chromaffin cells are known to exhibit Na channel-dependent action potentials, which under certain conditions are elicited by the depolarization resulting from cholinergic receptor stimulation [4]. Thus, the inhibitory effect of ibogaine on ACh-evoked secretion might involve

a block of Na channel spiking. Therefore, we tested whether ibogaine may act as a Na channel antagonist, i.e., have a tetrodotoxin (TTX)-like effect at an intermediate step following cholinergic stimulation. Fig. 2 shows that while 10 μ M ibogaine inhibited ACh-stimulated release, the sodium channel blocker tetrodotoxin (TTX) had no effect. The efficacy of TTX for sodium channels was confirmed by demonstrating its ability to inhibit veratridine-stimulated catecholamine release (Fig. 2). These data further demonstrate that low dose (10 μ M) ibogaine inhibition of cholinergically-stimulated catecholamine release is not sodium channel mediated.

The action of ibogaine on nicotinic receptor-mediated responses was further demonstrated using three different nicotinic agonists. Fig. 3 shows the dose-dependent inhibition by ibogaine of catecholamine release evoked by nicotine (10 μ M), ACh (100 μ M) and dimethylphenylpiperazinium (DMPP) (10 μ M). In these experiments, the cells were first preincubated with varying concentrations of ibogaine for 10 min. The ibogaine was removed during the subsequent stimulation with nicotinic agonist. The results in Fig. 3 show that ibogaine inhibits catecholamine release evoked by nicotine, ACh and DMPP with IC_{50} 's of 6.9, 8.6, and 11.0 μ M respectively, and that the inhibitory effects are not immediately reversible upon ibogaine washout.

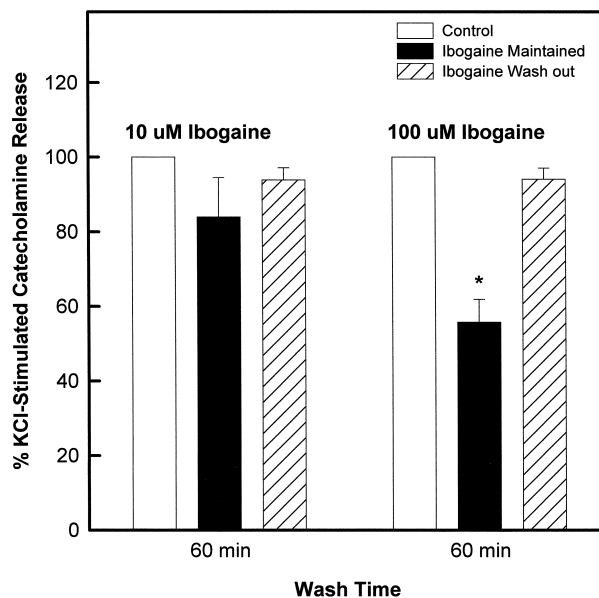


Fig. 5. Test of reversal of high dose ibogaine inhibition of KCl-stimulated catecholamine release. Cells were preincubated with either 10 μ M or 100 μ M ibogaine for 120 min, and subsequently subjected to a 60 min wash in which the appropriate dose of ibogaine was either present (■) or washed out (hatched bars). Catecholamine release was stimulated by incubating the cells in 70 mM KCl for 10 min in the presence (■) or absence (hatched bars) of ibogaine. Results are expressed as the % of control 70 mM KCl-stimulated release in the absence of ibogaine. Data represent the mean $\pm$ SEM of 3 independent experiments, each performed in triplicate. * indicates significant difference ($p < 0.05$) between control KCl-stimulated release (open bar) and that in the presence of 100 μ M ibogaine as determined by paired Student's *t*-test.

Prolonged effects of ibogaine on the dopaminergic system following an initial exposure have been reported in several studies [31,44]. To determine whether ibogaine exerted a long-term effect on stimulated catecholamine release in neuronal cell culture, chromaffin cells were pretreated with 10 μ M or 100 μ M ibogaine for 2 h and then washed several times to remove residual extracellular ibogaine. Cells were then placed in HEPES buffered solution for various time intervals before challenge with ACh. In parallel experiments, ibogaine was maintained throughout the protocol (solid bars). Following the removal of 10 μ M ibogaine for 10 min, ACh-stimulated catecholamine release was inhibited approximately 26%, as opposed to 69% inhibition with no washout. When the wash time was extended to 1 h or 19 h, the 10 μ M ibogaine pretreatment was completely reversed, i.e., it had no significant effect on ACh-stimulated release (Fig. 4A). When cells were pretreated with a higher ibogaine concentration (100 μ M), comparable to what occurs in the brains of animals within minutes after exposure to a typical dose [40,48], a prolonged inhibitory effect on ACh-stimulated release was observed. Following the removal of ibogaine for 10 min, 1 h and 19 h, ACh-stimulated catecholamine release contin-

ued to be inhibited by 73%, 55% and 30%, respectively (Fig. 4B).

We previously reported that high dose ibogaine can inhibit KCl-evoked catecholamine release from chromaffin cells [42]. Experiments parallel to those in Fig. 4 were therefore performed to determine whether the prolonged inhibitory effects following washout noted above were specific to cholinergic receptor-mediated release. Chromaffin cells were treated for 2 h with either 10 μ M or 100 μ M ibogaine and then washed several times to remove residual extracellular ibogaine. In control samples, the ibogaine was maintained during the wash. KCl-evoked release was inhibited by 44% when 100 μ M ibogaine was maintained during the 60 min wash. In contrast to the prolonged effect on ACh-evoked release, Fig. 5 shows that the inhibitory effect of high dose ibogaine (100 μ M) on KCl evoked release was largely reversed (6% inhibition, not significant) by the removal of ibogaine during the 60 min wash. The lower dose of ibogaine (10 μ M) had no significant inhibitory effect on KCl evoked release following an initial 2 h exposure, regardless of whether ibogaine was maintained or washed out.

To test the possibility of an ibogaine action mediated by kappa opioid receptors, the effects of the specific kappa opioid antagonist, norBNI, and the general opioid antagonist, naltrexone, on ibogaine inhibition of release was determined. Fig. 6 shows that preincubation of cultured chromaffin cells with either 1 μ M norBNI or up to 10 μ M naltrexone did not reverse the inhibitory effect of ibogaine on catecholamine release.

4. Discussion

Ibogaine has been reported to interact with a number of neuronal sites and to inhibit catecholamine release in the mesolimbic system [2,30,32] and in neuronal cell culture [42,43]. Mesolimbic catecholamine release is widely considered to be part of the reward pathway mediating positive reinforcement in drug addiction. Thus, ibogaine's putative anti-addictive properties may be related to its ability to inhibit catecholamine release. In the present study we have obtained results relevant to several postulated cellular mechanisms by which ibogaine may attenuate the catecholamine release response. These include a direct interaction with nicotinic acetylcholine receptors, a possible action at voltage-gated Na^+ or Ca^{2+} channels and a possible interaction with kappa opioid or sigma receptors. The evidence supports a selective action of ibogaine, at low concentrations ($< 10 \mu\text{M}$), at the nicotinic ACh receptor, most likely within the receptor cation channel. Ibogaine was found to produce a dose-dependent inhibition of catecholamine release evoked by three different nicotinic receptor agonists: nicotine, DMPP and ACh (Fig. 3). Furthermore, low doses of ibogaine ($< 10 \mu\text{M}$) selectively inhibited the nicotinic receptor pathway while not affecting

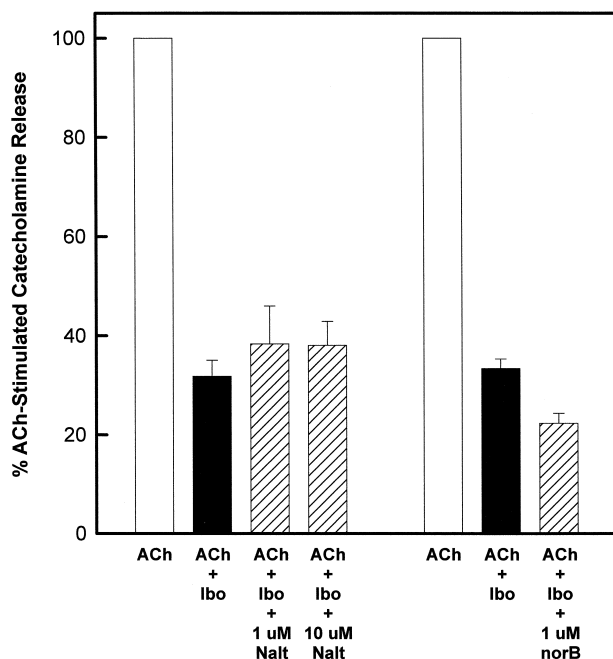


Fig. 6. Test for reversal by opioid receptor antagonists of ibogaine inhibition of ACh-stimulated catecholamine release. Cultured chromaffin cells were preincubated with the opioid antagonist naltrexone or norBNI for 10 min prior to and during a 10 min exposure to 10 μ M ibogaine. Cells were then challenged with 100 μ M ACh for 10 min in the continuing presence of ibogaine and indicated opioid receptor antagonist. Results are expressed as % of control ACh-evoked release in the absence of opioid ligands and represent the mean $\pm$ SEM of 3–4 independent experiments each done in triplicate. * indicates ACh-stimulated catecholamine release in the presence of ibogaine differs significantly from that in the presence of ibogaine + norBNI with a $p < 0.05$ as determined by paired Student's t -test.

release evoked by membrane depolarization with either elevated K^+ or with the Na channel agonist, veratridine. We have also shown results demonstrating a prolonged inhibitory action of high concentration (100 μM) ibogaine on ACh-mediated release that persisted for at least 19 h following ibogaine washout (Fig. 4).

The persistent effect of high ibogaine concentration on catecholamine release from chromaffin cells could be mediated by a number of sites including nicotinic ACh receptors, voltage-gated Na^+ and/or Ca^{2+} channels [42]. This effect is unlikely to be mediated by voltage gated channels since the inhibitory effects of high ibogaine concentrations (100 μM) on KCl-evoked release were largely reversed within 60 min of washout (Fig. 5). Since different nicotinic receptor subtypes are known to differ in their affinities for various pharmacological and toxicological agents, it is plausible that the persistent inhibitory effect of high dose ibogaine is mediated by one of the subtypes of nicotinic ACh receptor with a lower affinity for ibogaine.

The question of whether the persistent high dose ibogaine effects may result from a toxic effect on chromaffin cells needs to be considered. This is unlikely for the following reasons: (1) we have observed that high dose ibogaine does not affect basal catecholamine release [42] or ^{45}Ca uptake (unpublished result); (2) the high dose effects on ACh and K-evoked catecholamine release were largely reversible over a period of 1–19 h. If the high dose inhibitory effects were due to toxicity we would not expect reversibility of inhibition of K evoked release within 1 h; (3) we tested the effects of 2 h of 100 μM ibogaine on chromaffin cell viability using vital dye staining methods and found no increase in cell death in ibogaine exposed samples; and (4) the high dose effects we observe are inhibitory to release, while a toxic effect would normally cause a loss of semipermeability of the plasma membrane, with associated Ca^{2+} loading and enhancement of release.

The concentration range of ibogaine used in the present study (1–100 μM) is comparable to that reported in the brain of animals during a period of minutes to hours following a dose producing an anti-addictive action [20,40,48]. Within 10 s following iv administration of 10 mg/kg or at 10 min following sc administration of 20.9 mg/kg to mice, ibogaine concentrations in brain were reported to be of the order of 100 μM and subsequently declined with a half life of about 1 h [48]. Hough et al. [20] reported ibogaine levels in brain of about 20 μM at 1 h after administration of 40 mg/kg sc to rats. Thus, ibogaine may initially have multiple sites of action following the transient high brain concentrations and then have a selective action at the nicotinic receptor during its metabolism to lower brain concentrations. Our recent findings showing that high concentrations of ibogaine (100 μM) inhibit ACh, K and veratridine-evoked catecholamine release, while low concentrations (< 10 μM) have a selective action on nicotinic receptor-mediated release, are consistent with this model [42].

An action of ibogaine at the nicotinic ACh receptor is consistent with recent reports demonstrating in vivo inhibitory effects of ibogaine on nicotine-evoked dopamine release in the mesolimbic system of the rat [2,32]. Nicotine's stimulation of mesolimbic dopamine release is thought to be activated by nicotinic receptors in the ventral tegmental area (VTA) [13,36,37,47]. These VTA nicotinic receptors may be located on presynaptic glutamate terminals which enhance glutamatergic stimulation of dopaminergic neurons and/or on postsynaptic dopaminergic cell bodies which project to the nucleus accumbens. The nicotinic receptor subunits present on dopaminergic cell bodies include $\alpha 3$, $\alpha 4$, $\alpha 5$, $\beta 2$ and $\beta 3$ subtypes [11]. Those present on the glutamatergic terminals in the VTA may include $\alpha 7$, by analogy with nicotinic receptors present on glutamatergic terminals in other parts of the brain [33]. Since chromaffin cells are known to have $\alpha 3$, $\alpha 5$ and $\alpha 7$ nicotinic receptor subunits [9,14] it is plausible that ibogaine may be acting at one or more nicotinic receptor subtypes containing different combinations of these subunits.

Further support for ibogaine acting at the nicotinic receptor cation channel comes from the recent report of Badio et al. [1], showing that ibogaine inhibits nicotine- and carbamylcholine-induced ^{22}Na uptake in cultured rat pheochromocytoma (PC12) cells and human medulloblastoma TE671 cells. They also found that ibogaine blocks an antinociception effect in rats induced by the high affinity nicotinic agonist epibatidine. The in vitro block of ^{22}Na uptake in neuronal cell culture was interpreted as an action of ibogaine at a non-competitive site on the nicotinic receptor (i.e., the receptor cation channel) based on the inability of the ibogaine block to be overcome by increasing nicotine concentrations. This would be consistent with our earlier findings utilizing radiolabelled histrionicotoxin binding, where we reported that compounds containing an iboga ring within their structure, e.g., the vinca alkaloids, interact with the cation channel site within nicotinic receptors [35]. There is also evidence that ibogaine can interact with a non-competitive site within the NMDA receptor channel [40], and other drugs which interact with this site, e.g., MK-801 and phencyclidine (PCP), are also known to interact with the nicotinic receptor cation channel [5,41]. There is the distinct possibility that the mechanism of ibogaine's putative anti-addictive action involves sites within both the nicotinic and NMDA receptor cation channels in the mesolimbic system.

Catecholamine release from chromaffin cells, as in nerve terminals, is known to be triggered by calcium uptake through voltage-gated Ca^{2+} channels following membrane depolarization. Membrane depolarization can be achieved by elevating extracellular KCl or by activating Na^+ channels and/or nicotinic receptor channels. The lack of effect of low concentrations of ibogaine (< 10 μM) on catecholamine release evoked by depolarization with elevated KCl or by the Na channel agonist veratridine (Fig. 1)

would argue against an action of ibogaine at either voltage-gated Na^+ or Ca^{2+} channels. The possibility of Na^+ channels being part of the pathway of nicotinic receptor stimulated release that is blocked by ibogaine was further ruled out by the fact that the Na^+ channel blocker TTX had no effect on ACh-evoked release under identical conditions that release was blocked by ibogaine (Fig. 2). The effectiveness of TTX in blocking Na^+ channels in our system was confirmed by its ability to block veratridine-induced release (Fig. 2).

Several radioligand binding studies have demonstrated an interaction of ibogaine with kappa opioid receptors [15,46]. Kappa receptors are known to be present on chromaffin cells [8] and kappa agonists have also been shown to inhibit nicotine-induced calcium uptake and catecholamine release in this system [7,24]. We therefore tested whether the inhibitory effects of ibogaine on catecholamine release might be mediated by a kappa opioid receptor. If ibogaine was acting through a kappa receptor, then kappa opioid antagonists should prevent ibogaine's inhibitory effects. The opioid receptor antagonists did not affect ibogaine's inhibition of catecholamine release (Fig. 6). The lack of reversal of the inhibitory effects of ibogaine by 1 μM norBNI or 1–10 μM naltrexone would argue against the kappa opioid receptor as the site of ibogaine action in chromaffin cells. NorBNI at a concentration of 1 μM is more than adequate to block kappa receptors and has no significant effect on ACh evoked catecholamine release. We have found that norBNI concentrations of > 10 μM are directly inhibitory to ACh evoked release and thus could not be used to test for reversal of ibogaine's inhibitory effects.

Another possible site of ibogaine action is at sigma receptors. Ibogaine has been reported to interact with both sigma 1 and sigma 2 receptors subtypes with affinities compatible with anti-addictive levels of ibogaine in rodent brains [3,29,40]. Ibogaine has also been reported to alter sigma receptor-mediated dopamine release from mouse striatal tissue [45]. In chromaffin cells, sigma receptor agonists have been reported to non-competitively inhibit nicotine stimulated catecholamine release with a rank order of potency consistent with an interaction with sigma 1 receptors [38]. It is interesting that the benzomorphan opiates, e.g., cyclazocine, pentazocine, etc., are both kappa agonists and also sigma receptor ligands [22] and like ibogaine, have a selective non-competitive inhibitory action on nicotinic receptor mediated responses [38]. A plausible mechanism for the inhibitory actions of ibogaine would be an interaction within the nicotinic receptor ion channel, a location that may contain the sigma 1 receptor site or a structure closely resembling it. This would be consistent with the report of Paul et al. [38] which postulated that the sigma 1 receptor was within the nicotinic channel in chromaffin cells. We have also obtained results showing a selective action of cyclazocine, a sigma agonist, at the nicotinic receptor in chromaffin cells (unpublished

data). As with ibogaine (Fig. 1), pentazocine was shown to block nicotinic-, but not K^+ - or veratridine-evoked catecholamine release from chromaffin cells [38]. A mechanism of action of ibogaine within the nicotinic channel would also be consistent with the recent results of Badio et al. [1] with transformed chromaffin cells (PC12 cells) and with our earlier study [35] showing that compounds containing iboga ring structures (vinca alkaloids) bind within the nicotinic cation channel.

In summary, ibogaine has been shown to have a selective inhibitory action on nicotinic ACh receptor-mediated catecholamine release which appears to occur via a non-competitive interaction with the receptor cation channel. Since nicotinic receptors in the VTA are known to directly and indirectly modulate mesolimbic dopamine release as part of the brain's reward pathway, they will have to be considered along with other proposed sites (e.g., NMDA channels) [40] as part of the mechanism of the putative anti-addictive action of ibogaine. The ability of ibogaine to block nicotine-evoked catecholamine release could be relevant to the possible future utilization of it and related compounds in smoking cessation.

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